MicroGEM


Upper Wright Valley, Ross Sea Region - Antarctica
Source of ***forensic***GEM®

# *forensic*GEM® Sperm Lysis : validation of a rapid sperm lysis protocol for Y screening using the PDQeX Nucleic Acid Extractor and real time PCR

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## Introduction

The MicroGEM *forensic*GEM sperm lysis chemistry (formerly called ZyGEM sexcrime) has been validated for use in thermal cyclers and MicroGEM's PDQeX Nucleic Acid Extractor. This application note describes validation studies using the PDQeX instrument to prepare samples for Y screening. The same chemistry has been validated for use in thermal cyclers by the NY OCME and by DNA Solutions (poster presented at the ISHI 2019 meeting).

The *forensic*GEM sperm lysis kit uses MicroGEM's Acrosolv reagent to lyse sperm without the use of chemicals that inhibit qPCR, such as SDS, mercaptoethanol and DTT. When used with the PDQeX instrument, the reagents allow comprehensive lysis of sperm cells in 20 minutes. Since there are no transfer steps, the yield is maximized and the opportunities for mistakes and contamination are minimized. The protocol described in this note can be used to screen potential sexual assault evidence by following lysis with a Y chromosome-specific real time PCR assay or used directly for sperm lysis as part of a differential extraction procedure.

**NY OCME Protocol:** (https://www1.nyc.gov/assets/ocme/downloads/pdf/technical-manuals/protocols-for-forensic-str-analysis/zygem-one-step-sperm-lysis-of-sexual-assault-stains-or-swabs.pdf)

**MicroGEM Application Note**

## Materials and Methods

Small cuttings from vaginal swabs or cloth were cut and placed in PDQeX tubes. The sperm lysis reagents were mixed as shown in the following Mastermix (1x):

78 µL nuclease free water
10 µL Acrosolv
2 µL *forensic*GEM
10 µL Orange Plus Buffer

### *PDQeX Setup*

100 µL of the mastermix was pipetted into each PDQeX tube. Tubes were flicked to dislodge any bubbles and to fully immerse the material. Up to 24 PDQeX tubes can be loaded into the PDQeX simultaneously. Collection tubes were placed in the lower drawer and then the instrument was loaded with PDQeX tubes.

The following program was run for sperm lysis:

52°C for 5 minutes
75°C for 3 minutes
95°C for 3 minutes
110°C for 2 minutes

### *DNA Quantitation*

After extraction, the substrate remains in the PDQeX tube, and liquid DNA extract is passed into the collection tube ready for qPCR or STR profiling.

For the purpose of Y screening, qPCR was performed using a Promega Plexor® HY System on an Applied Biosystems 7500 Fast Real Time PCR System.

### *STR Profiling*

For samples amplified for STR loci, the Promega Powerplex® Fusion System was used. Approximately 1ng of template DNA was amplified for 28 cycles on an Applied Biosystems 9700. Separations were performed on an Applied Biosystems 3130xl.

### *Dilution Series Preparation*

A dilution series of sperm cells mixed with female epithelial cells was created using previously counted spermatozoa (Table 1). Epithelial cell count was based on prior qPCR data. The dilution series was extracted on the PDQeX in triplicate and then quantified using real time PCR.

| Sperm (per µL) | Epithelial Cells (per µL) |
|---|---|
| 400 | 370 |
| 50 | 370 |
| 5 | 370 |
| 1 | 370 |

**Table 1:** Liquid dilution series of sperm mixed with female epithelial cells.

### *Mock Casework Sample Preparation*

The dilution series described above was also used to create mock sexual assault samples. 100 µL samples from each of the dilution series were deposited onto sterile cotton swabs and dried overnight to mimic a vaginal swab. Small cuttings (the tip of the cotton swab ~3mm$^2$) were taken from each swab and extracted in the PDQeX.

To mimic a typical forensic casework sample, semen of unknown cell count was deposited on denim jeans with garden soil stains. The jeans were then stored at room temperature for one month. Two cuttings (~3mm$^2$ each) were taken from a portion of the jeans with obvious soil. An additional two cuttings were taken from an unsoiled area of the jeans.

## Results

### *Sensitivity Study Results*

The dilution series was extracted in triplicate and the average autosomal and Y quantifications are reported in Table 2.

| Sperm Count | Average of Auto (ng/µL) | Average of Y (ng/µL) |
|---|---|---|
| 400 | 4.424 | 3.736 |
| 50 | 3.550 | 0.534 |
| 5 | 2.204 | 0.025 |
| 1 | 2.243 | 0.008 |

**Table 2:** Real time PCR results from dilution series of sperm and epithelial cells from Table 1. The data shows the average concentration of DNA from three replicate extractions at each sperm cell count.

The qPCR data in Table 2 demonstrates that the PDQeX lyses sperm and epithelial cells in mixtures that can then be quantified using real time PCR to detect male DNA. The method is sensitive and ease of use makes it ideal for rapid evidence screening. This process also provided a good estimate of the male DNA to total gehomic DNA actually in the sample.

### *Mock Casework Results*

The data from the mock vaginal swabs (Table 3) created from the dilution series in Table 1 demonstrated that the PDQeX can extract DNA from mixtures of epithelial and sperm cells dried on cotton swabs.

| Sample | Auto (ng/µL) | Y Conc (ng/µL) |
|---|---|---|
| Swab_400 | 20.78 | 8.33 |
| Swab_50 | 8.513 | 0.81 |
| Swab_5 | 13.16 | 0.14 |
| Swab_1 | 6.390 | 0.025 |

**Table 3:** Real time PCR quantification data from dilutions of sperm in female epithelial cells dried onto cotton swabs.

The qPCR data from these samples was used to determine the amount of template added for STR amplification (Figure 1).

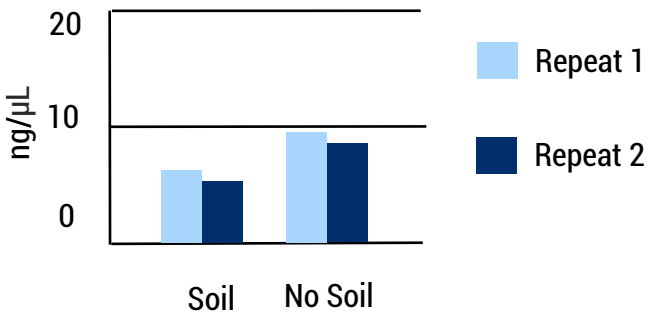


**Figure 1:** Y qPCR data from semen from two replicates at soiled and non-soiled areas of denim jeans.

### *STR Data*

The qPCR data from Figure 1 was used to calculate the template DNA input for STR amplification of the semen samples dried on denim. All four samples yielded full profiles upon amplification, indicating the qPCR data was indicative of DNA quantity and quality. Figures 2 and 3 are STR profiles from two of those samples.

## Conclusions

These validation studies confirm the efficacy of *forensic*GEM sperm to rapidly prepare samples for Y screening. Lysis of sperm and epithelial cells in mixtures can be quantified using real time PCR to detect male DNA. Further, this process provides a good estimate of male DNA to total genomic DNA. Finally, the real time PCR data from the soiled denim was useful in estimating DNA concentration for STR amplification, indicating that the extraction can handle common inhibitors such as soil and denim.

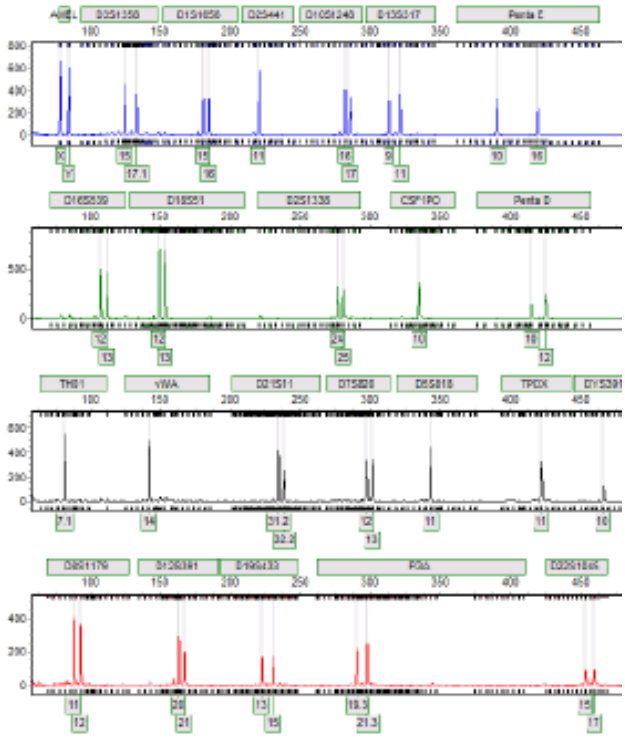

**Figure 2:** STR profile from semen deposited on a soiled area of denim jeans.

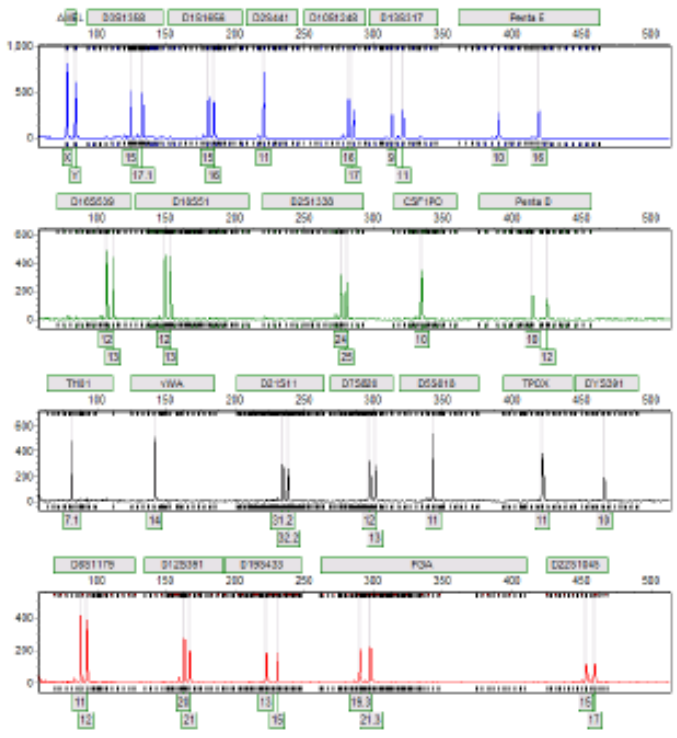

**Figure 3:** STR profile from semen deposited on an unsoiled area of denim jeans .